Background: The aim of this study was to investigate oxidative stress and thiol/disulfide status with a novel automated homeostasis assay in advanced non-small cell lung cancer (NSCLC). Methods: Thirty-five patients with advanced NSCLC, who had been newly diagnosed and previously untreated, and 35 healthy subjects were chosen for the study. We measured plasma total thiol (–SH+–S–S–), native thiol (thiol) (–SH), and disulfide (–S–S–) levels in the patients with NSCLC and the healthy subjects. The thiol/disulfide (–SH/–S–S–) ratio was also calculated. Results: Statistically significant differences between the patient group and the control group were detected for the thiol/disulfide parameters. The mean native thiol, total thiol, and disulfide levels were significantly lower in the group with advanced stage NSCLC. The cut-off value was 313 and 13.8 for native thiol and disulfide, respectively. Median overall survival (OS) was significantly shorter in patients with low native thiol and disulfide levels according to the cut-off value (respectively, \( P = 0.001; P = 0.006 \)). Native thiol, total thiol, and disulfide levels were correlated with Karnofsky performance status (KPS), OS, and age. Additionally, hierarchical regression analyses showed gender, KPS, lung metastases, and plasma native thiol levels were the determinants of OS in the final model. Conclusion: These results suggest that in advanced stage NSCLC, the native thiol, total thiol, and disulfide levels decrease, while the native thiol/disulfide ratio does not change. Low levels of thiol/disulfide parameters are related to tumor aggressiveness and may predict a poor outcome for patients with NSCLC.